REMARKS

Claims 29-45 are canceled without prejudice or disclaimer. Claims 1-28 were previously canceled. Claims 46-64 are added. In particular, the previously pending claims have been recast as claims 46-61 and are fully supported throughout the specification as filed including, e.g., the original claims. Added claims 62-64 are supported throughout the specification as filed including, e.g., page 6, lines 14-18.

It is respectfully submitted that the present amendment presents no new issues or new matter and places this case in condition for allowance. Reconsideration of the application in view of the above amendments and the following remarks is requested.

I. Priority

Applicants acknowledge with appreciation the Examiner's statement regarding the priority date of October 22, 2004 for claims 29-39 and 42-44. However, Applicants submit that the pending claims are also entitled to the Danish Application No. PA 2003 01562 filed October 23, 2003 and U.S. Provisional Application No. 60/515,000 filed October 28, 2003, of which the present application claims the priority or the benefit. Applicants request that the Examiner so acknowledge in the next Office Communication.

Applicants address the Examiner's contentions regarding the priority of claims 40-41 in response to the written description rejection, below.

II. The Rejection of Claims 29-44 under 35 U.S.C. 112, Second Paragraph

Claims 29-44 stand rejected under 35 U.S.C. 112, second paragraph as allegedly being indefinite in the recitation of "with more than xx% sequence identity." This rejection is respectfully traversed.

To expedite prosecution, Applicants have amended the claims to delete recitation of the objected-to phrase, thereby obviating the rejection.

For the foregoing reasons, Applicants submit that the claims overcome this rejection under 35 U.S.C. 112, second paragraph. Applicants respectfully request reconsideration and withdrawal of the rejection.

III. The Rejection of Claims 40-41 under 35 U.S.C. 112, First Paragraph (Written Description)

Claims 40-41 stand rejected under 35 U.S.C. 112, first paragraph as allegedly failing to comply with the written description requirement. The Examiner alleges that the specification fails to describe the limitation of storage for 30 minutes at 35°C and therefore the claims are rejected for introducing New Matter. This rejection is respectfully traversed.

Applicants respectfully submit that prior claims 40-41 are supported throughout the specification as filed, including, e.g., Example V, Stability in Detergent, which states at page 42, line 36 that the mixture to be assayed is incubated for 30 minutes at temperatures including 30°C and 35°C. Nevertheless, to expedite prosecution, the amended claims herewith delete recitation of this limitation, thereby obviating the rejection.

For the foregoing reasons, Applicants submit that the claims overcome this rejection under 35 U.S.C. 112, first paragraph (written description). Applicants respectfully request reconsideration and withdrawal of the rejection.

IV. The Rejection of Claims 29-39 and 42-43 under 35 U.S.C. 102(b)

Claims 29-39 and 42-43 stand rejected under 35 U.S.C. 102(b) as allegedly anticipated by Isono et al., USPN 3,655,570 ("Isono et al.") as evidenced by Isono et al. and Esaki et al., Arch. Microbiol., 161:110-115 (1994) ("Esaki et al."). In particular, in the outstanding and prior Office Actions, relying on Example 6/Table 5 of Isono et al., the Examiner states that Isono et al. teach an alkaline protease isolated from *Fusarium solani* (IFO 5232) that shows activity in a detergent composition. Esaki et al. is cited to support the proposition that Isono's *F. solani* alkaline protease has thermostability since an aminotransferase from *F. solani* has thermostability. The Examiner acknowledges that Isono et al. provide no characterization of the proteases isolated from *F. solani*, and states that no evidence was provided that any of the proteases isolated from *F. solani* were alkaline proteases. The Examiner further states that the evidence provided in Table 5 of Isono et al. was obtained using a culture supernatant, which would contain all proteases produced by the *F. solani*, which is the strain used by Applicants. Therefore, the Examiner concludes, the skilled artisan would believe, more likely than not, that the isolate of Isono et al. included the protease of SEQ ID NO: 2 now claimed by Applicants. This rejection is respectfully traversed.

The Isono et al. patent, which is assigned on its face to Takeda Chemical Industries, Ltd., discloses cultivation of strains of alkaline protease-producing microorganisms belonging to the genus Fusarium. *Fusarium solani* (IFO 5232) and *Fusarium sp.* S-19-5 (IFO 8884) are described

as typical microorganisms producing the alkaline protease. See generally, Isono et al., col. 2, lines 30-44. Applicants address each of these strains in turn.

Turning first to the *Fusarium sp.* S-19-5 strain, Isono et al. at Examples 1-4 describes culture conditions for inoculation with *Fusarium sp.* S-19-5 (IFO 8884) to obtain a crude enzyme powder, which is purified and characterized. The amino acid sequence of this alkaline protease is not disclosed in Isono et al.; however, as set forth in Applicants' prior Response, many years after the filing of the Isono et al. patent application, Takeda disclosed the amino acid sequence of the *Fusarium sp.* S-19-5 (IFO 8884) alkaline protease in U.S. Patent No. 5,543,322 and Morita et al., 1994, *Biosci. Biotech. Biochem*. 58(4): 621-626 (copy previously provided). The characteristics of the alkaline protease described in the '322 patent and Morita et al. are consistent with the characteristics of the alkaline protease described in Isono et al. Thus, persons of ordinary skill in the art would expect that the alkaline protease described in the '322 patent and Morita et al. is the same protease described in Isono et al. at Examples 1-3.

In addition, as also outlined in Applicants' prior response, according to the MEROPS protease database, alkaline proteases – such as those described in Isono et al. – belong to MEROPS Family S8. See http://merops.sanger.ac.uk/index.htm; Rawlings et al., 2008, MEROPS: the peptidase database. *Nucleic Acids Res.* 36: D320-D325 (copies previously provided). "Alkaline proteases" are a defined class also known as the Subtilisins. See Kumar and Takabi, 1999, Biotechnology Advances 17:561-594, in particular page 562, first full paragraph (copy provided herewith for the convenience of the Examiner). The group of microbial proteases historically called alkaline proteases and/or Subtilisins (Family S8) share a similar structure, and therefore, similar sequence.

However, one of skill in the art would also know that that not all proteases that are active in the alkaline range belong to the Family S8. Thus, although trypsin-like proteases – such as those that are the subject of Applicants' claims – may be active at alkaline pH, they nevertheless belong to an entirely different class of proteases than the Family S8 proteases, which include the Subtilisins or alkaline proteases. In fact, the claimed trypsin-like proteases belong to a different MEROPS family, Family S1. See also id. This is further exemplified in Applicants' specification as filed, which shows that the claimed *F. solani* trypsin-like protease (as well as another Fusarium trypsin-like protease, SP387) are different in substrate-specificity and inhibitor sensitivity than the subtilisin Savinase. See, e.g., Specification, Figures 1-2. Savinase is also identified in Table 4 of the Kumar review on alkaline proteases.

For at least this reason, a person of ordinary skill in the art would not believe that Isono et al.'s protease produced from *Fusarium sp.* S-19-5 (IFO 8884), which is an alkaline protease, is the same as the trypsin-like protease of SEQ ID NO: 2 of the present invention. Moreover, a comparison of the amino acid sequences of the Isono et al. alkaline protease from *Fusarium sp.* S-19-5 (IFO 8884), as evidenced by the '322 patent and Morita et al., and SEQ ID NO: 2 of the present application shows that the two sequences are not homologous.

Thus, the alkaline protease from Fusarium sp. S-19-5 (IFO 8884) does not teach or suggest the pending claims.

Turning now to the *Fusarium solani* (IFO 5232) strain, Isono et al. simply states that "[i]n the same manner as in Example 1," *F. solani* (IFO 5232) is cultivated, the culture is centrifuged to give supernatant fluid which is used as an enzyme solution, and enzyme activity with and without detergent is demonstrated from this alkaline protease-producing microorganism. See Isono et al., Example 6 and Table 5. In particular, Table 5 presents data that protease activity is measurable in an *F. solani* fermentation broth in the presence of the detergent LAS. However, Isono et al. do not purify or characterize the *F. solani* activity in any way.

In view of this disclosure regarding *F. solani* (IFO 5232), the Examiner concludes that:

> More importantly, the evidence provided in Table 5 of Isono et al was obtained using a culture supernatant, which would contain all proteases produced by the F. solani strain, the same strain used by Applicants. Thus, the skilled artisan would believe that, more likely than not, said isolate of Isono et al included the protease set forth by SEQ ID NO: 2 herein.

Office Action, page 5.

Applicants respectfully submit that the Examiner's conclusion is incorrect for at least the following reasons.

First, it is incorrect that "the evidence provided in Table 5 of Isono et al was obtained using a culture supernatant, which would contain ***all*** proteases produced by the F. solani strain." Id. (emphasis added). A person skilled in the art would not expect *F. solani* to necessarily produce *all* of its proteases at any one time. Gene expression in microbes is carefully regulated to fit the environment conditions and developmental stage of the culture. Various literature references demonstrate this differential regulation of proteases in fungi. For example, Bye et al., Arch. Microbiol. 189:81-92 (2008) (copy submitted herewith for the Examiner's convenience), shows that the production of different subtilisins in the same fungus varies with growth substrate. Bye et al., Introduction, Figure 1 and p. 91. ("[T]he results presented here show significant differences in

substrate specificity and regulation of subtilisin Pr1-like proteases…."). As another example, Paterson et al., Microbiology, 140:185-189 (1994) (copy submitted herewith for the Examiner's convenience) describes a protease that seems only to be produced when the pathogenic fungus is presented with a very specific substrate. See Paterson et al. generally, Abstract. Paterson also notes that "[t]here have been many reports of protease production being effected in fungi by exogenous protein under conditions of nutrient deprivation." Id., Discussion. Accordingly, one of skill in the art would recognize that only the proteases induced by growth of *F. solani* under the culture conditions described by Isono et al. in Example 1 (as referenced in Example 6) would be present in the supernatant fluid of Example 6. It would be incorrect to assume that *all* possible proteases of *F. solani* would be present in the supernatant fluid, let alone all at measureable levels.

Second, the culturing conditions of *F. solani* (IFO 5232) in Example 6 of Isono et al. are in fact quite different from Applicants' culturing conditions in the instant specification. Isono et al. uses a complex procedure in fermentation tanks while Applicants ferment in a shake flask for seven days at 25°C. See generally, Example 6 as evidenced by Example 1 of Isono et al. and Example 1 of the instant specification. Moreover, the Isono et al. medium includes

> 500 parts by volume of a liquid medium, composed of 5 percent defatted soybean meal, 5 percent glucose, 2 percent sodium dihydrogen phosphate, and adjusted to pH 7 , is dispensed in a fermenter (its capacity being 2,000 parts by volume),

(Isono et al., Example 1, col. 4, lines 59-66) while Applicants' medium includes

- 30g wheat bran, and
- 45 mL of the following solution:
  - 0.18g Yeast Extract,
  - 0.045 g KH_2PO_4,
  - 0.0225g $MgSO_4.7H_2O$,
  - 0.675 g glucose, 45 ml tap water

(Specification, p. 39, line 35 to p. 40, line 4). In particular, the nitrogen source of the Isono et al. cultivation (soybean meal) is different than Applicants' nitrogen source (yeast extract and wheat bran). One of skill in the art would recognize the importance of the nitrogen source in regulating protease production. As outlined above, gene expression in fungi is carefully regulated and culture conditions have an effect on protease production. One of skill in the art would recognize that the differences in nitrogen source, amongst other factors, would lead to different proteases being produced under the different culturing conditions of Isono et al. as compared to Applicants' conditions.

Finally, given that the culture conditions of *F. solani* (IFO 5232) in Isono et al. Example 6 are "[i]n the same manner" as those of Example 1, one of skill in the art could infer that the culture supernatant of *F. solani* likely includes the homolog of the alkaline protease isolated and characterized from *Fusarium sp.* S-19-5 (IFO 8884). Although Isono et al. provide no information as to which of the many proteases that *F. solani* could potentially produce is responsible for the effect seen in Table 5, one of skill in the art could reasonably infer that it is the homolog of the alkaline protease isolated and characterized from *Fusarium sp.* S-19-5 that actually provides this effect. This is further supported by the identification in Takeda's later '322 patent of *F. solani* as potential source for homologs to the cloned alkaline protease of IFO 8884. See '322 patent, col. 3, lines 4-9. As stated above, the protease produced from *Fusarium sp.* S-19-5 (IFO 8884) is not the same as the protease of SEQ ID NO: 2.

For all of these reasons, one of skill in the art would not conclude that the protease produced from *F. solani* (IFO 5232) under the conditions outlined in Isono et al. Example 6 is the same as the protease of SEQ ID NO: 2. *Thus, the alkaline protease from F. solani (IFO 5232) does not teach or suggest the pending claims.*

For the foregoing reasons, Applicants submit that the claims overcome this rejection under 35 U.S.C. 102(b). Applicants respectfully request reconsideration and withdrawal of the rejection.

V. The Rejection of Claim 44 under 35 U.S.C. 103

Claim 44 stands rejected under 35 U.S.C. 103 as allegedly being unpatentable over Isono et al. in view of Okuda et al., US Publication 2004/0002432 ("Okuda et al.") for reasons of record. This rejection is respectfully traversed.

As explained in detail above, Isono et al. does not teach or suggest the claimed proteases.

Okuda et al. disclose a detergent composition comprising an alkaline protease and one or more other enzymes. However, Okuda et al. does not teach or suggest detergent compositions comprising the proteases of the present invention.

For the foregoing reasons, Applicants submit that the claims overcome this rejection under 35 U.S.C. 103. Applicants respectfully request reconsideration and withdrawal of the rejection.

VII. Conclusion

In view of the above, it is respectfully submitted that all claims are in condition for allowance. Early action to that end is respectfully requested. The Examiner is hereby invited to

contact the undersigned by telephone if there are any questions concerning this amendment or application.

All required fees were charged to Novozymes North America, Inc.'s Deposit Account No. 50-1701 at the time of electronic filing. The USPTO is authorized to charge this Deposit Account should any additional fees be due.

Respectfully submitted,

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